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Culture of *Spirulina platensis* in human urine for biomass production and O₂ evolution*

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Abstract: Attempts were made to culture *Spirulina platensis* in human urine directly to achieve biomass production and O₂ evolution, for potential application to nutrient regeneration and air revitalization in life support system. The culture results showed that *Spirulina platensis* grows successfully in diluted human urine, and yields maximal biomass at urine dilution ratios of 140~240. Accumulation of lipid and decreasing of protein occurred due to N deficiency. O₂ release rate of *Spirulina platensis* in diluted human urine was higher than that in Zarrouk medium.

Key words: *Spirulina platensis*, Human urine, Biomass production, O₂ evolution, Life support system

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INTRODUCTION

For long distance manned space missions, biological life support systems (BLSS) have to be introduced into the life support system to achieve regeneration of nutrient matter (Volker and Frank, 2001). Recently, many kinds of subsystems within BLSS have been proposed and experimented on solely or jointly, which include mainly higher plant, aquatic organisms (Volker and Frank, 2001), microorganisms and microalgae (Gros *et al.*, 2003). Microalgae were incorporated in many BLSS because of their high growth rate, simple cultivation and relatively easy collection (Gitelson and Rodicheva, 1993). Among the microalgae, *Spirulina platensis* is the most commonly employed because of its rich nutrition and potential for substitution for conventional food (Ciferri and Tiboni, 1985).

In Micro Ecological Life Support System Alternative (MELSSA), a first attempt was made to use

treated waste, mainly including feces and human urine, to culture *Spirulina platensis* (Gros *et al.*, 2003). In the system, three steps biological pre-treatment of the waste was applied, so that the complete system became somewhat complicated. Some organic matters in feces, such as fiber, are inefficient for bio-treatment. Human urine contains minimal organic matters, but much inorganic matter, such as N, P and K (Larsen *et al.*, 2001), and so, is ideal culture medium for the photoautotrophic *Spirulina platensis*. We reported here our attempts at culturing *Spirulina platensis* in human urine solely and directly to achieve nutrient regeneration and air revitalization.

MATERIALS AND METHODS

Spirulina platensis 439 was obtained from the Freshwater Algae Bank of the Chinese Academy of Sciences (CAS). Culture medium used in this study includes Zarrouk medium (ZM) (Gòdia *et al.*, 2002), synthetic human urine (SHU) (Gordon, 1982) and real human urine (RHU). ZM (1000 ml) is composed of

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18.0 g NaHCO₃, 2.5 g NaNO₃, 0.5 g K₂HPO₄, 1.0 g K₂SO₄, 1.0 g NaCl, 0.04 g CaCl₂, 0.08 g Na₂EDTA, 0.2 g MgSO₄·7H₂O, 0.01 g FeSO₄·7H₂O and 1.0 ml trace elements (TE). TE (g/L): H₃BO₃ 2.86; (NH₄)₆Mo₇O₂₄ 0.02; MnCl₂·4H₂O 1.8; Cu₂SO₄ 0.08; ZnSO₄·7H₂O 0.22. The culture medium pH was adjusted to 8.2 by 1 mol/L NaOH solution. SHU (1000 ml) was composed of 0.5 g CaCl₂·2H₂O, 4.12 g K₂HPO₄, 0.47 g MgCl₂·H₂O, 0.29 g KCl, 4.83 g NaCl, 1.55 g NH₄Cl, 2.37 g Na₂SO₄, 13.34 g urea, 1.0 g creatinine and 0.65 g sodium citrate (pH 6.8).

A bubble column photobioreactor with total culture volume of 1200 ml was designed and used to culture *Spirulina platensis*. Culture condition was set as follows light intensity 444.4 w/m², aeration rate 1.8 L/min, illuminating time 14.0 h/d and temperature 30 °C. *Spirulina platensis* in ZM and log phase was collected and inoculated into different culture medium to start the culture process, during which, de-ionized water was added daily to compensate for water loss by evaporation.

Water, protein, lipid, total chlorophyll, carotenoid, dissolved oxygen (DO) and ash were analyzed following standard methods (APHA *et al.*, 1995; Sullivan and Carpenter, 1993). Metallic elements, including K, Na, Ca, Mg and Fe were determined using ICP-AES (Inductively coupled plasma-atomic emission spectrometry).

DO increasing rate in ZM and 180-diluted RHU were measured in closed photobioreactor. Before starting the experiments, O₂ was removed by purging with N₂ for 15 min at rate of 6 L/min. CO₂ (100 ml) was then injected into the bottom of the reactor. Recirculation (2 L/min) was started to avoid biomass sedimentation and improve mass transfer. Inoculated biomass concentration was about 0.6 g/L, light intensity 444.4 w/m² and temperature 30 °C.

Linear growth rate (v) (Ogbonna *et al.*, 1995) was introduced to evaluate the growth rate of *Spirulina platensis*, and can be calculated according to the equation: $v=(DW_t-DW_i)/t$, where v is the linear growth rate, t is the culture time, DW_i and DW_t is the dry weight at the beginning and end of the linear growth phase respectively; each growth curve was calculated after logistic curve fitting. The maximum value of the curve is then defined as the maximum productivity (P_{max}), used to evaluate the maximal biomass concentration.

RESULTS AND DISCUSSION

Results of batch culture of *Spirulina platensis* in 180-diluted RHU, 180-diluted SHU and commonly used ZM are shown in Fig.1. *Spirulina platensis* grew successfully and yielded similar culture results in 180-diluted RHU ($v=0.266$ g/(L·d), $P_{max}=2.32$ g/L) and SHU ($v=0.236$ g/(L·d), $P_{max}=2.40$ g/L), and their growth curves showed no statistical different (95% confidence level). Moreover, their main compositions were on the same level (Table 1).

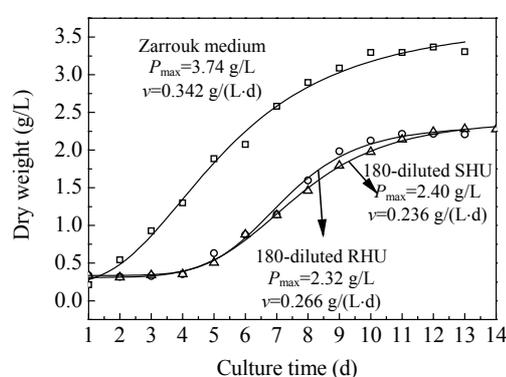


Fig.1 Culture results of *Spirulina platensis* in 180-diluted RHU, 180-diluted SHU and ZM

Table 1 Main composition of *Spirulina platensis* cultured in 180-diluted RHU, 180-diluted SHU and ZM

Composition (100 g dry wt)	180-diluted RHU	180-diluted SHU	ZM
Water (g)	4.98	5.23	4.23
Protein (g)	32.40	34.78	67.20
Lipid (g)	20.43	17.58	11.93
Total chlorophyll (mg)	1429.19	1408.56	1399.68
Carotenoid (mg)	227.99	269.49	337.76
Ash (g)	4.42	4.50	6.50
K (mg)	229.31	245.66	564.52
Na (mg)	457.18	483.49	723.44
Ca (mg)	332.25	336.19	65.84
Mg (mg)	726.35	648.87	1337.68
Fe (mg)	5.79	19.45	37.08

Except for 3 d additional lag phase, *Spirulina platensis* in these three culture media showed similar culture characteristics, growing exponentially in log phase, and stabilized in stationary phase. P_{max} in 180-diluted RHU and SHU was lower than that in ZM

(3.74 g/L), which might have resulted from the nutrient shortage. The composition of *Spirulina platensis* in 180-diluted RHU was rich in lipid (20.43%), but deficient in protein (32.40%), in contrast with that in ZM which were 11.93% and 67.20% correspondingly. 180-diluted RHU (C:N:P molar ratio: 20.04:21.6:1) was apparently deficient in C but rich in N, in comparison with ZM (C:N:P molar ratio: 74.66:10.25:1). During batch-culture, CO₂ from air can be used as C source. Due to evaporation loss of NH₄-N (Carvalho et al., 2004), N shortage appeared (N:P molar ratio: 2.8:1) when the culture process entered into log phase, and subsequently synthesis of amino acids and protein was inhibited.

Fig.2 showed the effect of dilution ratio on the growth of *Spirulina platensis*. P_{\max} gets its peak within the range of about 140~240. Low P_{\max} in low dilution ratio may result from inhibition effects of high NH₄-N concentration (Carvalho et al., 2004). And low P_{\max} in high dilution ratio may due to scarcity of nutrient matters.

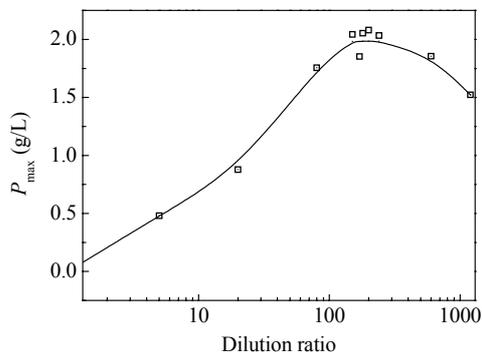


Fig.2 Dilution ratio on maximal biomass (P_{\max}) of *Spirulina platensis* cultured in diluted SHU

DO increasing in ZM and in 180-diluted RHU in the closed photobioreactor are shown in Fig.3. DO rose sharply at first, but finally stabilized near their maximal values (ZM: 7.01 mg/L; 180-diluted RHU: 6.80 mg/L). Michaelis-Menten equation (Ritchie and Prvan, 1996) could describe the effect of dissolved CO₂ concentration (S_{CO_2}) on DO increasing rate (v_{DO}):

$$v_{\text{DO}} = v_{\text{DOmax}} S_{\text{CO}_2} / (K_{S_{\text{CO}_2}} + S_{\text{CO}_2})$$

where $K_{S_{\text{CO}_2}}$ is Michaelis-Menten constant. At the

beginning, S_{CO_2} was high enough, and the equation is simplified to $v_{\text{DO}} = v_{\text{DOmax}}$, which means a zero-order increase in DO as was the case in both culture media within the initial 60 min. DO increasing can be described as: $d_{\text{DO}}/dt = K_{\text{DO}}$, where K_{DO} is the reaction constant and t is the reaction time. Then, K_{DO} in ZM and 180-diluted RHU was calculated to get the value of 49.30 and 62.75 $\mu\text{g}/(\text{L}\cdot\text{min})$ respectively, which show a higher O₂ release rate in 180-diluted RHU.

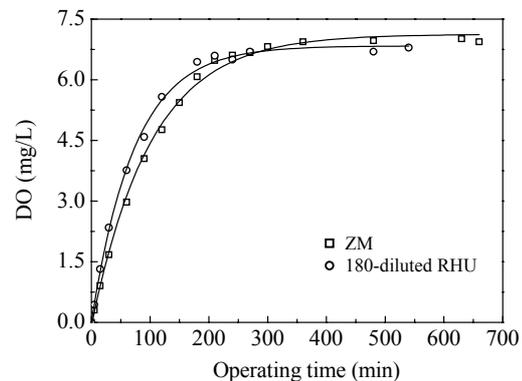


Fig.3 Variation of DO in closed photobioreactor with ZM and 180-diluted RHU as culture medium

CONCLUSION

Spirulina platensis was successfully grown in diluted RHU to achieve biomass production and O₂ evolution. Thus, it is possible to use *Spirulina platensis* to regenerate nutrient matter, and at the same time, to assimilate CO₂ and release O₂ within the life support system.

References

- APHA (American Public Health Association), AWWA (American Water Works Association), WEF (Water Pollution Control Federation), 1995. Standard Methods for Water and Wastewater Examination. Washington DC.
- Carvalho, J.C.M., Francisco, F.R., Almeida, K.A., Sato, S., Converti, A., 2004. Cultivation of *Arthrospira* (*Spirulina*) *platensis* (cyanophyceae) by fed-batch addition of ammonia chloride at exponentially increasing feeding rates. *J. Phycol.*, **40**:589-597.
- Ciferri, O., Tiboni, O., 1985. The biochemistry and industrial potential of *Spirulina*. *Annu. Rev. Microbiol.*, **89**:503-526. [doi:10.1146/annurev.mi.39.100185.002443]
- Gitelson, J.I., Rodicheva, E.K., 1993. Self-Restoration a Specific Feature of Biological Life Support Systems. SAE

- Technical Paper Series No. 961495, 26th International Conference on Environmental Systems 8-11, California.
- Gòdia, F., Albiol, J., Montesinos, J.L., Pérez, J., Creus, N., Cabello, F., Mengual, X., Montras, A., Lasseur, C., 2002. MELISSA: a loop of interconnected bioreactors to develop life support in Space. *J. Biotechnol.*, **99**:319-330. [doi:10.1016/S0168-1656(02)00222-5]
- Gordon, R., 1982. *Essentials of Human Physiology*, 2nd Ed. Year Book Medical Publishers, Chicago.
- Gros, J.B., Poughon, L., Lasseur, C., Tikhomirov, A.A., 2003. Recycling efficiencies of C, H, O, N, S and P elements in a biological life support system based on microorganisms and higher plants. *Adv. Space Res.*, **31**(1):195-199. [doi:10.1016/S0273-1177(02)00739-1]
- Larsen, T.A., Peters, I., Alder, A., Eggen, R., Maurer, M., Muncke, J., 2001. Re-engineering the toilet for sustainable wastewater management. *Environ. Sci. Technol.*, **35**(9):192A-197A.
- Ogbonna, J.C., Yada, H., Tanaka, H., 1995. Light supply coefficient: a new engineering parameter for photobioreactor design. *J. Ferment. Bioeng.*, **80**(4):369-376. [doi:10.1016/0922-338X(95)94206-7]
- Ritchie, R.J., Prvan, T., 1996. A simulation study on designing experiments to measure the K_m of Michaelis-Menten kinetics curves. *J. Theor. Biol.*, **178**:239-254. [doi:10.1006/jtbi.1996.0023]
- Sullivan, D.M., Carpenter, D.E., 1993. *Methods of Analysis for Nutrition Labeling*. AOAC International, Arlington, VA, p.85-104.
- Volker, B., Frank, P., 2001. Aquatic modules for bioregenerative life support system based on the C.E.B.A.S biotechnology. *Acta Astronaut.*, **48**:287-297. [doi:10.1016/S0094-5765(01)00025-X]



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